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Co-infection with *Neisseria mucosa* in a patient with tuberculous otitis media

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ABSTRACT

Tuberculous otitis media (TOM) is a rare manifestation caused by *Mycobacterium tuberculosis* with low incidence rates among extrapulmonary tuberculosis cases. Diagnosis is often delayed because of the presence of several clinical manifestations and the high prevalence of secondary bacterial infections. Few reports have attributed secondary bacterial infections in patients with TOM to commensal *Neisseria*. Thus, understanding the pathogenic mechanisms and clinical features of commensal *Neisseria* is important, considering its recent presentation as an infection-causing pathogen. *Neisseria mucosa* is a commensal inhabitant in humans and is generally considered non-pathogenic but can cause infection in rare cases. Here, we report an atypical secondary infection caused by *Neisseria mucosa* in an 81-year-old woman with TOM being treated for pulmonary tuberculosis. Direct purulent otorrhea smear microscopy revealed no acid-fast bacilli using Ziehl-Neelsen staining, whereas the phagocytosis of gram-negative cocci by white blood cells was confirmed using Gram staining. Otorrhea culture revealed the growth of *N. mucosa*. Subsequently, *M. tuberculosis* infection in the otorrhea was identified using a culture-based method. Vigilance is critical for the early detection of TOM to prevent further complications. This report raises awareness regarding TOM and provides insight into the pathogenicity of *N. mucosa* in otitis media.

1. Introduction

Although the incidence of *Mycobacterium tuberculosis* (*MTb*) infections in Japan has recently decreased, its prevalence remains high, with a notification rate of 9.2 cases per 100,000 population reported in 2021 (Tuberculosis Surveillance Center, 2022). Tuberculous otitis media (TOM) is a rare manifestation of *MTb* infection, with a very low incidence rate among extrapulmonary tuberculosis infections. Diagnosis is often delayed because of the presence of several clinical manifestations and high prevalence of secondary bacterial infections. The *Neisseria* genus includes pathogenic and non-pathogenic species. Although some infections have been attributed to commensal *Neisseria*, these organisms are generally non-pathogenic and are rarely considered in routine clinical investigations unless they are isolated from clinically significant sites. Vigilance is critical for the early detection of TOM to prevent further complications. Additionally, few reports have attributed secondary bacterial infections in patients with TOM to commensal *Neisseria*. Thus, understanding the pathogenic mechanisms and clinical features of commensal *Neisseria* is important, considering its recent presentation as an infection-causing pathogen. Here, we report an atypical secondary infection caused by *Neisseria mucosa*.

2. Case report

An 81-year-old woman presented with 4 kg weight loss at 3 months after the onset of anorexia. Chest computed tomography revealed small nodules distributed randomly throughout both lungs. Ziehl-Neelsen

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Abbreviations

CT	computed tomography
MGIT	mycobacteria growth indicator tube
MTb	*Mycobacterium tuberculosis*
PTB	pulmonary tuberculosis
TOM	tuberculous otitis media
WBC	white blood cell
ZN	Ziehl-Neelsen

(ZN) sputum smear microscopy was positive, and polymerase chain reaction analysis revealed the presence of *MTb*. The patient was referred to the emergency department and complained of recent-onset lumbago and shivering. She was hospitalized and treated for suspected miliary tuberculosis. She had been treated with mecobalamin after developing facial palsy one week prior, which was evaluated as Grade IV according to the House Brackmann grading system. Her medical history included atrial fibrillation, degenerative L3 spondylolisthesis, osteoarthrosis, and an unruptured cerebral aneurysm, which was managed conservatively. The patient was administered acetaminophen as required. On admission, blood tests showed an elevated C-reactive protein level (73.2 mg/L), slightly elevated liver enzyme levels (alanine aminotransferase, 32 U/L; aspartate aminotransferase, 57 U/L), low sodium level (129 mEq/L), and a normal white blood cell (WBC) count (4.1×10^9/L). Lumbar spine CT demonstrated degenerative changes but no bone destruction or abscess formation. Some clinical specimens (such as sputum, blood, cerebrospinal fluid, pleural fluid, and urine) were cultured in a mycobacteria growth indicator tube (MGIT) using the BACTEC MGIT 960 system (BD Biosciences, Franklin Lakes, NJ, USA) to isolate *MTb* and accurately diagnose miliary tuberculosis. The sputum culture detected *MTb* susceptible to isoniazid, rifampicin, ethambutol, rifabutin, streptomycin, levofloxacin, ciprofloxacin, and kanamycin; however, the other clinical specimens did not reveal *MTb*. She was first administered isoniazid, rifampicin, and ethambutol, and pyrazinamide was added after improved liver enzyme levels were confirmed. In addition to anti-tuberculosis therapy, she was administered oral sodium tablets because of suspected hyponatremia caused by the syndrome of inappropriate antidiuretic hormone, which gradually improved her serum sodium level. ZN sputum smear microscopy results were negative after 1 week of hospitalization.

After 2 weeks of anti-tuberculosis therapy, the patient demonstrated painless purulent otorrhea in the left ear without hearing loss, and blood tests showed a slightly elevated C-reactive protein level. Otoscopic examination revealed external auditory canal stenosis, which did not allow for observation of the tympanic membrane. Suction for otorrhea was performed for pathogen detection. Microscopic examination using ZN and Gram staining revealed negative ZN staining and phagocytosis of gram-negative cocci by WBCs (Fig. 1). Ofloxacin ear drops (0.3%) were administered, along with repeated suction discharge, because the otorrhea persisted despite anti-tuberculosis therapy. Subcultures were performed on 5% sheep blood agar (BD Biosciences) in 5% CO_2 at 37 °C and using MGIT at 35 °C. Pure bacterial colonies were observed on sheep blood agar after 24 h of incubation. The colonies were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper; Bruker Daltonics GmbH & Co. KG, Leipzig, Germany) as *Neisseria subflava* with an unsatisfactory score (1.88) for species confirmation. Therefore, additional biochemical tests (ID test HN-20 rapid; Nissui Pharmaceutical, Tokyo, Japan) were performed. The bacterium was positive for nitrate, and the species was identified as *N. mucosa* (biotype code; 7027012). The identification was confirmed using 16S rRNA sequencing. Antimicrobial susceptibility testing was performed using the broth microdilution method based on Clinical and Laboratory Standards Institute testing methods and breakpoints for

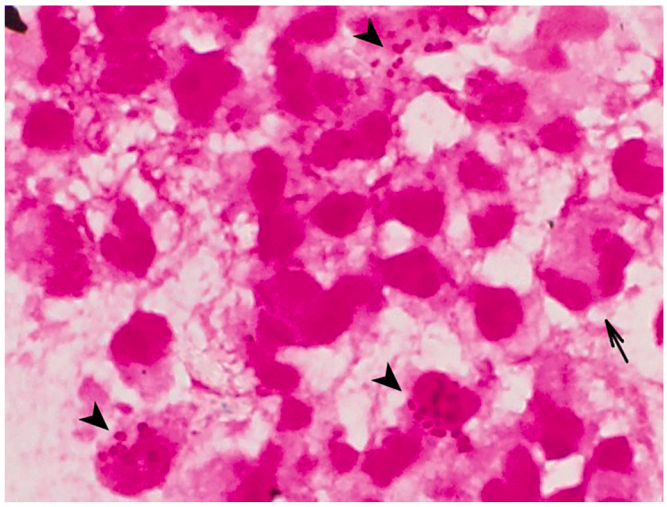

Fig. 1. Gram staining of the otorrhea showing phagocytosis of gram-negative cocci (1000 × magnification) (arrowheads) gram-negative cocci; (arrow) white blood cell.

Neisseria meningitidis, which showed minimum inhibitory concentrations of ceftriaxone (<0.12 μg/mL) and meropenem (<0.25 μg/mL) in the susceptible category and of penicillin (2 μg/mL), ampicillin (1 μg/mL), trimethoprim-sulfamethoxazole (0.25/4.75 μg/mL), and levofloxacin (0.06 μg/mL) in the resistant or intermediate category. At one week after ofloxacin treatment, the patient's clinical symptoms resolved. Subculture of purulent otorrhea with MGIT revealed *MTb* after 16 days of incubation. After the initial phase of treatment with the four anti-tubercular agents, the patient received a maintenance phase of treatment with isoniazid and rifampicin. Sputum culture negativity for *MTb* was confirmed after 7 weeks of anti-tuberculosis therapy, and the patient was transferred back to the introductory medical institution.

3. Discussion

Here, we report an atypical secondary infection in a patient with TOM caused by *N. mucosa*. TOM is a rare manifestation (Akkara et al., 2014) observed in less than 2% of patients with known active PTB and approximately 0.06% of all chronic otitis media cases (Skolnik et al., 1986). Otorrhea, which is particularly refractory to standard antibiotic treatment, is a clinical manifestation of TOM. Additionally, abundant and/or pale granulations are considered an important feature of TOM (Yaniv, 1987; Windle-Taylor and Bailey, 1980; Singh, 1991). However, TOM exhibits various clinical manifestations, which typically differ from the classical description (Yaniv, 1987; Cho et al., 2006; Vaamonde et al., 2004). Multiple tympanic membrane perforations are described as a classic feature; however, some reports indicate that this feature is more likely to be observed as a single perforation (Skolnik et al., 1986). Facial palsy, which can be caused by tuberculous mastoiditis, is also described a feature of TOM and occurs more frequently in children than in adults. Although the reported incidence rates vary from approximately 10 to 40%, the rate is considered to be higher than that in non-tuberculous chronic otitis media (Cho et al., 2006; Vaamonde et al., 2004; Windle-Taylor and Bailey, 1980; Singh, 1991). (Singh, 1991) reported that the facial nerve recovery rate was the same between surgical and non-surgical groups, which is dependent on early diagnosis and treatment with anti-tuberculosis therapy. Anti-tuberculosis therapy, using a combination of multiple antibiotics, is the standard treatment for TOM (Nahid et al., 2016). Often, antibiotic treatment alone is sufficient but may be supplemented with surgery in some cases (Cho et al., 2006; Singh, 1991). In our case, the patient's symptoms of otitis media were resolved with ofloxacin ear drops, anti-tuberculosis therapy, and suctioning discharge. Microbiological examination of the discharge, such as

smear microscopy and acid-fast bacilli culture, may be used to identify *MTb*; however, positivity in extrapulmonary tuberculosis is low (Cho et al., 2006; Vaamonde et al., 2004; Windle-Taylor and Bailey, 1980) because of the low mycobacterial counts. Histopathological examination of the tissue may further aid in detecting TOM (Cho et al., 2006; Vaamonde et al., 2004; Windle-Taylor and Bailey, 1980; Singh, 1991). Despite this, diagnosing TOM is often difficult. When detecting TOM, it is important to consider its differential diagnosis and remain vigilant, even if the microbiological examination of *MTb* is negative and bacteria other than *MTb* are detected. A high rate of secondary bacterial infections has been reported in TOM, further complicating the diagnosis. The common causative genera are *Staphylococcus*, *Pseudomonas*, *Proteus*, and *Streptococcus* (Yaniv, 1987; Windle-Taylor and Bailey, 1980; Manigandan et al., 2013), which typically cause otitis media in adults (Mofatteh et al., 2018; Verhoeff et al., 2006). In the present case, Gram staining of the otorrhea revealed phagocytosis of gram-negative cocci by WBCs. We initially assumed this bacterium to be *Moraxella catarrhalis*. Although rarely detected in adults, *M. catarrhalis* is widely recognized as the major causative agent of otitis media (Celin et al., 1991; Murphy and Parameswaran, 2009). However, subcultures identified the bacterium as *N. mucosa*. A literature review of 21 cases in 2014 attributed endocarditis to *N. mucosa* (Pilmis et al., 2014). Additionally, *N. mucosa* infection is implicated in several unusual cases of meningitis, septicemia, empyema, genital infections, urinary infection, botryomycosis, arthritis, and peritonitis (Humbert and Christodoulides, 2019; Awdisho and Bermudez, 2016). Caution is warranted in interpreting older reports because the species identification in previous reports may vary from the current classification, and recent studies of ribosomal multi-locus sequence typing suggested that *Neisseria macacae* and *Neisseria sicca* should be considered the same species as *N. mucosa* (Bennett et al., 2013). However, knowledge regarding the pathogenicity of commensal *Neisseria* species is limited. Several genes are shared among pathogenic and commensal *Neisseria*, which serve as reservoirs of virulence genes (Marri et al., 2010; Lu et al., 2019). In this case, *MTb* and *N. mucosa* were simultaneously detected in the otorrhea. Gram staining revealed the phagocytosis of *N. mucosa*, indicating infection. Additionally, serious otorrhea observed in patients with TOM can progress to purulent otorrhea because of secondary bacterial infections (Sens et al., 2008). The importance of *Neisseria* species in otitis media is known, and even nonpathogenic species synergistically contribute to or cause disease in the presence of other coinfecting species (Laufer et al., 2011). Therefore, we hypothesized that the phagocytosis of *N. mucosa* could be the result of secondary bacterial infections induced by TOM. However, this study was limited to clinical interpretations from a single patient. The hypothesis must be confirmed in a larger patient population.

Break points and antimicrobial susceptibility testing methods for *N. mucosa* have not been provided by the Clinical and Laboratory Standards Institute. Some patients have been successfully treated with beta-lactams or fluoroquinolones (Pilmis et al., 2014; Awdisho and Bermudez, 2016); however, commensal *Neisseria* have differing susceptibilities, and the antimicrobial resistance profiles have been explored (Goytia et al., 2021; Raisman et al., 2022). Topical antibiotics can deliver high concentrations of a drug to affected sites. Nishiike et al. (2000) described the effects of combining anti-tuberculosis therapy with 2% kanamycin ear drops; although this report included only six cases, the observations may indicate that ofloxacin treatment was effective against TOM as well as secondary bacterial infections. Our isolated *MTb* was susceptible to levofloxacin. In this case, the patient was successfully treated, and the purulent otorrhea improved. However, the suitability of this treatment is uncertain because the treatment for commensal *Neisseria* is unknown. Standard break points and antimicrobial susceptibility testing methods should be established.

In conclusion, we reported a rare case of TOM with *N. mucosa* coinfection. A laboratory bacterial diagnosis of TOM may be difficult because the positivity of acid-fast bacilli staining and culture is insufficient. Additionally, secondary bacterial infections may provide misleading results during early identification using Gram staining and/or culture. Although *N. mucosa* is often considered as a commensal of the oral and nasopharyngeal cavities in humans, it was involved in a secondary TOM infection in this case. This case report raises awareness of TOM and provides insights into the infectious potential of *N. mucosa* in otitis media.

Authors' contributions

T.H.: conceptualization, data curation, investigation, writing – original draft. K.S. Y.G. M.S. T.U.: writing – review & editing. Y.I. Y.M.: supervision, writing – review & editing. D.S. H.M.: investigation, writing – review & editing. All authors revised the drafts and approved the manuscript for publication.

Informed consent

Informed consent was obtained from the patient for the publication of this case report.

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Declaration of competing interest

None.

Authorship statement

All authors meet the ICMJE authorship criteria.

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